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Free radical scavenging activity in methanolic leaf extract of *Santalum album*

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ORIGINAL RESEARCH ARTICLE	ABSTRACT
ARTICLE INFORMATION <i>Article history</i> Received: 08 December 2013 Revised: 17 December 2013 Accepted: 22 December 2013 Early view: 30 December 2013 <i>*Author for correspondence</i> E-mail: yahyakhan28@gmail.com Mobile/ Tel.: <i>Keywords:</i> Antioxidants DPPH TLC Free radical.	Objectives: India has a rich in medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value. The aim of the present study to investigate the antioxidant constituents from the natural source from the leaves of <i>Santalum album</i>. Materials & Methods: Attempts were also made to extract phenolic antioxidant using aqueous and methanolic extracts were screened for antioxidant activity. The Antioxidant activity of constituents in aqueous and methanol extract was evaluated by DPPH free radical scavenging activity. Results: Pre-treatment of test animals with <i>Hemidesmus indicus</i> (75 & 100 mg/kg i.p) reduced the catalepsy score in haloperidol treated mice. Both the doses i.e. 75 & 100 mg/kg (i.p.) of <i>Hemidesmus indicus</i> showed protective effects against haloperidol-induced catalepsy. Conclusion: <i>Santalum album</i> is potent source of antioxidants further research is needed to isolate antioxidant from it.

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INTRODUCTION

Antioxidants in biological system have multiple functions including defense against oxidative damage caused by the action of reactive oxygen species (ROS). ROS such as superoxide radical (O_2^-), hydroxyl radical (OH), peroxide radical (ROO) and nitric acid radical are generated in living organisms during excessive metabolism and involved in extensive damage to cells that leads to age related degenerated diseases, cancer and wide range of other human diseases. Several synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertbutyl hydroquinone (TBHQ) are commercially available and are currently in use because of their carcinogenicity of synthetic antioxidant. There is preference to develop effective antioxidants of natural origin (Brand –Williams et al., 1995). The utilization of herbs, herbal extracts or plant derived pure chemicals to treat disease is a therapeutic modality, which has stood the test of time (Ansari and Inamdar, 2010). The natural antioxidant phenolic compounds play a key role in antioxidative defense mechanism in biological system and act as free radical scavengers. So now-a-days attention has turned on to natural antioxidant because the use of synthetic antioxidant has been falling off due to their suspected action as a cancer inducer (Adhikari et al., 2010; Morakinyo et al.,

2012; Sahoo et al., 2012). In India people have used sandal wood medicinally as remedy of many ailments. The *Santalum album* has shown the antioxidant activity. Satalbic acid derived from the oil *sacuminatum* R.BR. restricted the growth of *Collectotrichum*, *Fusarium* and certain plant pathogenic bacteria. Early *hawaiiensis* leaves and bark of *Santalum album* used as a remedy for dandruff. So based on the fact our study is designed to evaluate the antioxidant activity of *Santalum album* leaves. Except for the roots the entire plant is used as an anti-inflammation, antibacterial and anthelmintic (Sharma et al., 2001).

MATERIAL AND METHODS

Fresh and disease free leaves of *Santalum album* were collected from the campus of Government Institute of Science, Aurangabad, India. Collected leaves were washed with distilled water and then put it for drying under shade. After drying the dried leaves were grinded in fine powder using mechanical mixer.

Preparation of Extract

10 gm of powder of *Santalum album* leaves were soaked in 100 ml of 80 percent methanol, with shaking for a period of 24 h. After that sample were extracted using Whatman filter paper no.1 and the filtrate was used for the assay. Different

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concentrations were prepared for methanolic extract as 5%, 10%, and 15% in 80% methanol.

Chemicals and reagent

DPPH (1, 1-Diphenyl 1-2 picrylhydrazyl), ascorbic acid and Folin-Cio-calteu reagent was purchased from Dodal Enterprises, Aurangabad, India. All the chemicals used including the solvent were of analytical grade.

Preparation of solution of DPPH (1, 1-Diphenyl 1-2picryl hydrazyl)

1.5 mg of DPPH was weighed and dissolved in 5 ml methanol. Since DPPH is light sensitive so the solution was covered in aluminum sheet. After checking its absorbance at 517 nm, it was found to be less than one. 900µl of DPPH was added to 100µl extract and ascorbic acid then incubated at 25°C for 30 min followed by its absorbance at 517 nm was checked with the UV- spectrophotometer. Scavenging activity was determined applying following formula:

$$[(\text{DPPH abs} - \text{sample abs})/\text{DPPH abs}] \times 100$$

Antioxidant bioassay

In antioxidant bioassay three extract solutions were prepared as stock solution from the stock solution another type of solution was prepared with different conc. The total volume of this solution was 500 µl. Another five solution with different conc. were prepared as discussed previous these five solution of 50, 100, 150, 200, 250 µl/ml was also reused as stock solution (Table 1). Ascorbic acid was used as control which inhibits the free radicals. Solution of ascorbic acid was prepared with the same method of extract.

Table-1. Scavenging activity of *Santalum album* leaves.

S. No.	Conc. of extract	% Scavenging activity	
		Plant extract	Ascorbic acid (control)
1	50 µl	0.29	0.35
2	100 µl	0.37	0.41
3	150 µl	0.43	0.47
4	200 µl	0.48	0.51
5	250 µl	0.49	0.54

Table 2. R_f values of *S. album* extract

Sr. no.	<i>Santalum album</i> extracts (10%)	
	R_f values in cm	Mean of R_f values
1	0.60	0.61
2	0.65	
3	0.59	

Thin layer chromatography

TLC analysis was used for the detection of compounds. 2 ml of methanolic leaves extract was used for each spot. A pre-coated plate of silica gel 60F254 (Merck) and mobile phase chloroform: methanol: ethyl acetate (8.0: 1.5: 1.0) were used. The extracts fractionated by TLC and were identified by its R_f values using standard values.

RESULT

DPPH free radical has strong electron attracting ability from antioxidant. The graph showed that the conc. of methanolic extract is directly proportional to percentage scavenging activity of *S. album*. The minimum scavenging activity was shown at the lowest conc. 50 µl/ml (32.25%). The order of five scavenging activity is 50<100<150<200<250 µl/ml. Ascorbic acid was used as control.

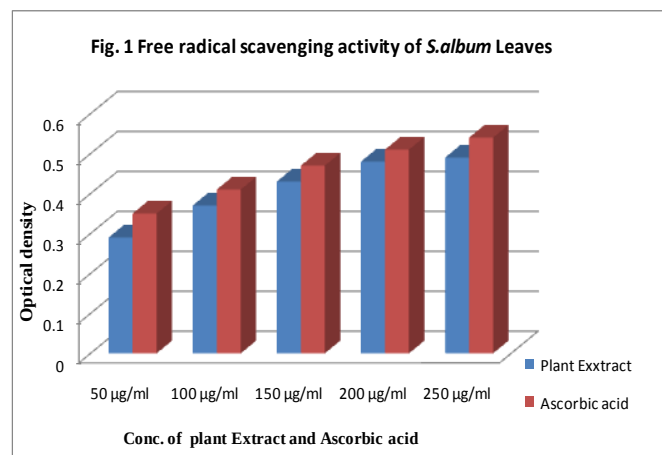


Figure 2. Free radical scavenging activity of *S. album* leaves.

DISCUSSION

It has been recognized that the flavonoid and phenolic compounds show antioxidant activity on human nutrition and health. The mechanisms of action of flavonoid and phenolic compounds are through scavenging process (Kessler et al., 2003; Cook and Samman, 1996). The TLC of methanolic

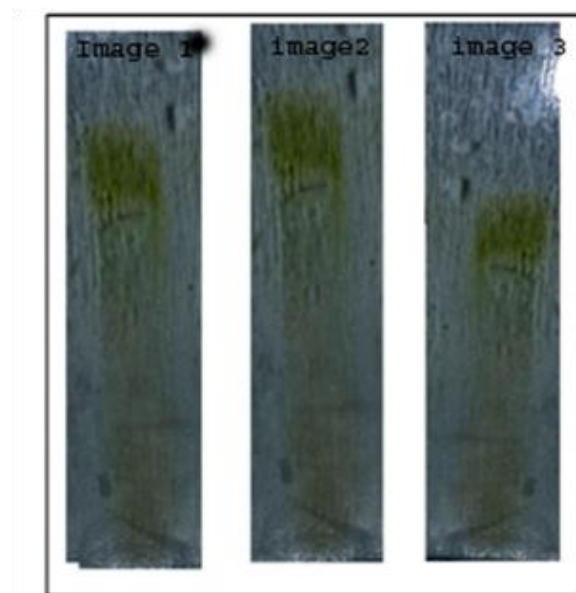


Figure 2. TLC of methanol extract of *S. album*

extract of *S. album* R_f closely related to the values obtained by Lie Ken Jie (2003) i.e. R_f 0.65. DPPH is one of the few stable organic nitrogen free radicals, which has been widely used to determine the free radical scavenging ability of the various samples (Brand –Williams et al 1995). The present study indicated that the free radical scavenging activity of methanolic extract posses marked scavenging activity by DPPH. Phenolic compounds are a class of antioxidant which acts as free radical terminators (Shahidi and Wanasundara,

1992). The antioxidant potential of the plant is due to the presence of compounds of phenolic, flavonoid and polyphenolics which are significantly inhibited by the free radicals that causes oxidative stress. That mean the leaves of *Santalum album* are the potent source of antioxidant agents. They also consist of antioxidizing compounds.

CONCLUSION

Santlun album is potent source of antioxidants and further research is needed to isolate antioxidant from it.

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